

Exposure to a theta-burst patterned magnetic field impairs memory acquisition and consolidation for contextual but not discrete conditioned fear in rats

B.E. McKay, M.A. Persinger*, S.A. Koren

Behavioral Neuroscience Laboratory, Laurentian University Sudbury, Ontario, P3E 2C6, Canada

Received 19 April 2000; received in revised form 2 August 2000; accepted 11 August 2000

Abstract

Preceding or immediately following fear-conditioning rats were exposed for 30 min to either a sham field, one of two symmetrical (sine-wave 7, 20 Hz) magnetic fields or to one of two complex magnetic fields whose waveforms were modeled after salient electrophysiological patterns within either the hippocampal formation (theta-burst) or the amygdaloid complex (burst-firing). The magnetic fields were presented in successive 2 s intervals through each of the three spatial planes and then simultaneously within all three planes. Field strengths ranged between 0.5 and 1 microTesla. Only the group exposed after the conditioning to the theta-burst (hippocampal) magnetic fields displayed evidence of forgetting, as inferred by their marked attenuation of freezing behavior, during contextual extinction 24 h later. This powerful treatment explained 75% of the variance in the extinction scores. Behavioral responses to the discrete conditioned stimulus were not affected. These findings are consistent with the involvement of the hippocampus in learned fear to contextual stimuli but not to discrete auditory stimuli and suggest that physiologically relevant stimuli may be delivered to the brain by weak, complex magnetic fields whose intensities are ubiquitous within modern environments. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Magnetic fields; Conditioning; Fear responses; Complex wave forms; Theta bursts

We have pursued the hypothesis that the brain functions as an electromagnetic neuromatrix. From this perspective: (1) all behaviours are the products of neuroelectromagnetic patterns associated with neurochemical activity and (2) brain space is immersed in complex time-varying electromagnetic fields that can be diffuse or constrained within non-adjacent spatial increments. For an environmental stimulus from natural (e.g. geomagnetic) or synthetic (electronic) sources to intercalate with the neuromatrix, the temporal and spatial characteristics of processes inside and outside of the brain must be congruent. The results of the present experiments showed that an electromagnetic pulse, whose intrinsic shape and interstimulus interval had been specifically designed to influence an electrophysiological process involved with learning, can evoke qualitatively conspicuous changes in behavior.

Maintained immobility by *Rattus norvegicus* while in the presence of contextual (non-specific or background) cues or

of discrete (tone) cues previously associated with an aversive event has been employed as an inference of learning [1]. Extensive research indicates that contextual and discrete classical conditioning involve different pathways within the brain [4]. Lesions of the hippocampus selectively impair acquisition and consolidation of contextual fear conditioning but minimally influence the acquisition and consolidation of auditory-cued fear behavior [10]. On the other hand, functional lesions of the amygdala impair acquisition and consolidation of all fear-related behavior [10]. Consequently fear conditioning to contextual or to discrete stimuli provides a behavioral paradigm by which contributions from the hippocampus and amygdala may be functionally dissociated.

During learning many amygdaloid neurons exhibit burst firing [15] whereas hippocampal pyramidal neurons display three to five pulse-bursts within the range of theta rhythms [8]. Synaptic facilitation or long term potentiation (LTP), a major electrophysiological correlate of long term memory, can be easily induced in vivo when 'natural patterns' are applied through direct electrical stimulation [2]. We

* Corresponding author. Fax: +1-705-671-3844.
E-mail address: f1200002@nickel.laurentian.ca
(M.A. Persinger).

reasoned that pre-conditioning and post-conditioning application of weak complex magnetic fields, similar to prominent hippocampal electrophysiological patterns, should selectively impair the acquisition and the consolidation of contextual fear conditioning.

Ninety-nine male Wistar strain albino rats served as subjects. Animals were obtained from Charles River (Quebec) and were 8–12 months old. Rats were housed in groups of 2–3 per cage in standard colony housing. Ambient temperature was maintained at $20 \pm 1^\circ\text{C}$. The light–dark cycle was 12:12 with photophase onset at 07:30 h local time. Food and water were available ad libitum. All conditioning and testing took place during the mid to late photophase.

Contextual and discrete conditioning as well as contextual extinction testing were conducted in a $28 \times 20 \times 20$ cm modified Plexiglas and aluminum operant chamber. The raised floor of the chamber consisted of 18 steel rods spaced 1.5 cm apart. Each rod was connected to a unit designed to deliver scrambled electric shocks. A 6 cm diameter speaker was mounted in one lateral wall and was connected to a frequency generator programmed to emit a specific tone. To facilitate removal of animals, a non-conducting frame whose dimensions approximated those of the chamber was inserted into the chamber prior to conditioning and testing each animal. Ambient sound intensity was approximately 50 dB. Extinction testing of the discrete stimulus was assessed in another room in a novel apparatus (a $33 \times 21 \times 23$ Plexiglas chamber with acoustic equipment mounted in one end). All apparatus was cleaned and dried before each animal was trained or tested.

Magnetic fields were applied in a room that had not been employed for conditioning. The computer equipment utilized to generate the magnetic fields converted a series of values ranging from 0 through 255 to a voltage ranging from -5 to $+5$ V. Neutral polarity was represented by the value 128 while negative and positive polarities were represented by values less than 128 and greater than 128, respectively. This series of values, if plotted against their sequence in the computer file, graphically depicted the waveform of the magnetic field. A Zenith XT personal computer with a port latency of $140 \mu\text{s}$ propagated the values in the waveform file to a custom constructed, digital-to-analogue converter that transformed the file into the appropriate voltage.

The apparatus that emitted the magnetic fields as a function of the voltage-graded input was a $30 \times 30 \times 30$ cm Plexiglas box with 50 ohm solenoids affixed perpendicularly to the center of each external face of the cube (six solenoids total). The solenoids were connected to the digital-to-analogue converter. The mechanical component from each solenoid had been replaced with a 15×0.5 cm steel rod to focus the weak-intensity magnetic fields. For this application the digital-to-analogue converter included a solid-state switching device (switching time in the order of nanoseconds) which enabled successive activation of opposing solenoid pairs (one north pole, one south pole) for 0.5 s each and

culminated with the activation all three solenoid pairs for 0.5 s (see Fig. 1). As a result, the field was rotated through three-dimensional Cartesian space every 2 s (e.g. 0.5 Hz) and then through all three planes simultaneously. These rotations, completed every 8 s, occurred continuously throughout the 30 min treatment period. The bottom one-third of the apparatus had been filled with corncob bedding to ensure the animal's resting position was in the center of the applied magnetic field.

Five treatment conditions were employed for the present study. Subjects were exposed to sham conditions, to one of the two complex magnetic field patterns, or to one of two sinusoidal magnetic field patterns. The first complex pattern was a theta burst stimulation pattern (maximum intensity about 500 nT) designed to mimic the firing parameters of hippocampal pyramidal cells during learning and to induce long-term potentiation or LTP in hippocampal slices [12]. This pattern consisted of five-pulse bursts at 100 Hz separated by 140 ms (e.g. theta rhythm). The second complex magnetic field pattern was a burst-firing pattern [11] modeled after recordings of amygdaloid activity in epileptic patients; its average intensity was approximately 900 nT. The two sinusoidal patterns had frequencies (maximum intensities in parentheses) of 7 Hz (400 nT) and 20 Hz (900 nT) and were employed as intensity controls for the theta burst stimulation and burst-firing pulses. Rats that received sham conditions (<10 nT) were placed in the treatment apparatus with only the digital-to-analogue converter activated.

Analysis of variance (ANOVA) was employed as the primary statistical tool. Post-hoc analyses were completed with the Scheffe ($P < 0.01$) test. All analyses were completed using SPSS software on a VAX 4000 computer. Eta-squared values were employed as indicators of effect size. It is defined as the amount of variance explained in the dependent measure, the immobility scores, by manipulations of the independent variable, magnetic field patterns.

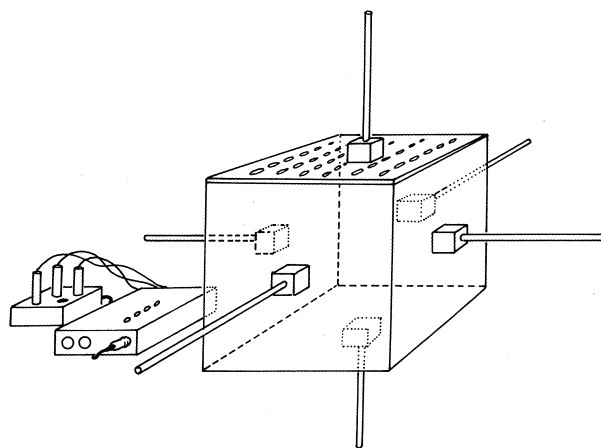


Fig. 1. Drawing of the exposure apparatus showing the positions of the pairs of modified solenoids along each spatial dimension. See body of text for details.

In Experiment I, 31 rats were trained in the contextual conditioning paradigm. Each animal was placed in the conditioning chamber and received four unsignaled footshocks (1-s, 0.5 mA) at 60 s intervals subsequent to an initial 2 min habituation period. The rat was removed from the conditioning apparatus immediately following the last footshock presentation and placed in the treatment room. Animals were randomly assigned to each of the five treatment groups (sham, hippocampal pattern, amygdaloid pattern, 7 Hz sine wave, or, 20 Hz sine wave). Subsequent to 30 min of magnetic field (or sham) exposure the rats were returned to their home cages. Freezing to contextual cues was assessed approximately 24 h later when the animals were returned to the conditioning apparatus and tested singly. The rats' behaviours were scored nominally for the presence or absence of freezing via a time-sampling procedure for 1 s every 8 s throughout the 8 min extinction test [6]. The number of freezing observations was converted to a percentage of total observations.

One-way analysis of variance indicated a statistically significant difference between treatment conditions for the proportion of freezing behaviours during contextual extinction testing ($F(4, 26) = 19.45$, $P < 0.001$, eta-squared = 75%). Post hoc analysis revealed the source of the effect to be the significantly reduced freezing-to-context for rats exposed to the theta burst stimulation magnetic field relative to all other treatment conditions that did not differ significantly from each other. The results are shown in Table 1. Pre-existing group differences in motoric activity, as defined by the numbers of forward movements of the head and forepaws over the midline of the chamber, were not evident ($F(4, 26) = 1.07$, $P = 0.39$).

In Experiment II, 30 rats were exposed for 30 min to either sham, burst-firing or theta-burst patterned magnetic fields prior to contextual conditioning. Because the two sinusoidal magnetic field treatments did not affect contextual extinction testing in Experiment I they were excluded from additional experiments. All conditioning parameters were identical to Experiment I. One-way analysis of variance demonstrated no statistically significant group

differences ($F(2, 27) = 2.28$, $P = 0.12$). The means and standard errors of the mean (SEM) are also shown in Table 1.

In Experiment III, 21 rats were conditioned to a discrete auditory stimulus. Following a 2 min pre-shock period these animals received four pairings of a 10 s auditory stimulus (2000 Hz, 90 dB) that terminated with the onset of a 1-s 0.5 mA footshock. The inter-pairing interval was 60 s. Rats were immediately relocated to the treatment room and exposed for 30 min to either the sham-field or to one of the two complex magnetic fields. Rats were tested for their recall of the discrete stimulus in the novel environment approximately 24 h later. Rats were placed in this environment for a total of 10 min which consisted of a 2 min pre-tone period and an 8 min tone presentation period. Freezing was scored during the tone presentation using the same procedure outlined for Experiment I. A one-way analysis of variance showed there were no statistically significant differences between the treatment groups for the duration of freezing behavior during extinction to the discrete stimulus ($F(2, 18) = 1.12$, $P = 0.35$).

Experiment IV was procedurally identical to Experiment III except magnetic fields were applied for 30 min prior to discrete conditioning and 17 rats were employed as subjects. There were no statistically significant differences between the three groups in freezing behavior during extinction ($F(2, 14) = 0.50$, $P = 0.62$). Differences in pre-tone freezing immediately prior to tone extinction as a function of treatment group were not significant statistically ($F(2, 15) = 0.02$, $P = 0.98$). Differences in spontaneous locomotor activity in all rats ($n = 47$) receiving pre-conditioning magnetic field treatment were not evident ($F(2, 44) = 1.80$, $P > 0.05$).

The results of these four experiments have shown that weak complex magnetic fields, patterned after the temporal parameters that elicited LTP in hippocampal slices [12], interfered with the memory of the contextual cues but not the discrete cues after fear conditioning when the rats were tested 24 h later. The effect was strongest when the rats were exposed to this specific field, but not to a less symmetric burst-firing pattern, during the 30 min after the acquisition. Exposure to the hippocampal pattern prior to conditioning attenuated freezing, i.e. exhibited a mild interference with contextual memory. However the effect was ambiguous statistically.

Sinusoidal magnetic fields, a popular temporal structure in many studies involving bioelectromagnetic effects, had no statistically significant influence upon the behavioral response to the conditioned contextual cues. Temporally symmetrical (sine-wave or square wave) magnetic fields may require higher intensities to evoke significant behavioral changes because they may be more dependent upon induction of electrical currents within susceptible structures or processes. That the sine wave magnetic fields were presented at the same intensity as the complex patterned fields yet did not affect this type of memory suggests wave-

Table 1

Means and SEM for percent freezing to contextual stimuli in rats exposed to sham conditions, one of two complex magnetic fields, or one of two sinusoidal magnetic fields for 30 min immediately following or preceding the conditioning session^a

Treatment	Post-Conditioning		Pre-Conditioning	
	Mean	SEM	Mean	SEM
Sham	93.3	1.77	81.2	4.6
Burst-Firing	77.1	6.00	80.5	5.4
Theta Burst	55.8**	3.65	65.8	7.0
7 Hz Sine-wave	95.8	1.71		
20 Hz Sine-wave	89.2	1.65		

^a Asterisks indicate a difference from all other context-conditioned groups, ** $P < 0.01$.

form structure rather than waveform intensity may sometimes be the critical factor mediating these effects of weak magnetic fields.

We suggest that powerful behavioral effects evoked by complex patterns of weak (500 to 1000 nT) magnetic fields are mediated through non-classical processes such as resonance interaction [5] or transfer of information [9]. We also assume that the probability of the intercalation of these complex magnetic fields with the electromagnetic patterns within the brain, the neuromatrix, may be strongly influenced by the application geometry. By successively applying these complex fields through each of the spatial planes and then simultaneously through all three dimensions, the intercalation within the neuromatrix of the moving rat may have been enhanced.

Low-intensity complex magnetic fields have been reported to simulate the activity of opioid agonists [14]; traditional introduction of opioid agonists can alter locomotor activity [13]. However, because group differences in motoric activity were not evident either immediately following magnetic field treatment or prior to extinction testing 24 h later in the present study, the likelihood that our observations were confounded by indirect effects upon motor activity is minimal. Interference with the capacity to express the freezing response was also not likely because the evidence of contextual fear but not discrete fear was attenuated by theta burst stimulation. Together these findings suggest a specific amnesic impairment rather than a motoric modulation and that the effects were not strongly dependent upon opioid-mechanisms.

The neuroanatomical pathways essential for acquisition, consolidation, and expression of learned fear to both contextual and discrete stimuli have been clearly defined [3]. The primary difference between these well-characterized pathways is the inclusion of the hippocampus in contextual fear conditioning, a structure required for integrating polymodal environmental stimuli [10]. Impairment of CA1 long-term potentiation in freely behaving animals has been shown to impair hippocampal-dependent memory processes [7]. Our application of a complex magnetic field pattern, whose equivalent when applied as electric current by electrodes has been shown to raise baseline field potentials in CA1 cells in vitro [12], may have randomly potentiated synapses indirectly through the putative calcium resonance model [5]. The resulting representation of the experience may have been quite different or incongruent with the actual veridical features of the learning event.

The authors wish to thank Lee S. Stewart for his technical assistance. This research was supported by the Laurentian University Neuroscience Group.

- [1] Bolles, R.C., Species-specific defense reactions and avoidance learning, *Psychol. Rev.*, 77 (1970) 32–48.
- [2] Dobrunz, L.E. and Stevens, C.F., Response of hippocampal synapses to natural stimulation patterns, *Neuron*, 22 (1999) 157–166.
- [3] Fendt, M. and Fanselow, M.S., The neuroanatomical and neurochemical basis of conditioned fear, *Neurosci. Biobehav. Rev.*, 23 (1999) 743–760.
- [4] Kim, J.J. and Fanselow, M.S., Modality-specific retrograde amnesia of fear, *Science*, 256 (1992) 675–677.
- [5] Lednev, V.V., Possible mechanism for the influence of weak magnetic fields on biological systems, *Bioelectromagnetics*, 12 (1991) 71–75.
- [6] Maren, S., De Oca, B. and Fanselow, M.S., Sex differences in hippocampal long-term potentiation (LTP) and pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning, *Brain Res.*, 661 (1994) 25–34.
- [7] Moser, E.I., Krobort, K.A., Moser, M.B. and Morris, R.G., Impaired spatial learning after saturation of long-term potentiation, *Science*, 281 (1998) 2038–2042.
- [8] Otto, T., Eichenbaum, H., Weiner, S.I. and Wible, C.G., Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation, *Hippocampus*, 1 (1991) 181–192.
- [9] Persinger, M.A., Metaphors for the effects of weak, sequentially complex magnetic field, *Percept. Mot. Skills*, 85 (1997) 204–206.
- [10] Phillips, R.G. and LeDoux, J.E., Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, *Behav. Neurosci.*, 106 (1992) 274–285.
- [11] Richards, P.M., Persinger, M.A. and Koren, S.A., Modification of activation and evaluation properties of narratives by weak complex magnetic field patterns that simulate limbic burst firing, *Int. J. Neurosci.*, 71 (1993) 71–85.
- [12] Rose, G.M., Diamond, D.M., Pang, K. and Dunwiddie, T.V., Primed burst potentiation: lasting synaptic plasticity invoked by physiologically patterned stimulation, In H.L. Haas and G. Buzaki (Eds.), *Synaptic Plasticity in the Hippocampus*, Springer-Verlag, Berlin, 1988, pp. 96–98.
- [13] Sills, T.L. and Vaccarino, F.J., Individual differences in the feeding and locomotor stimulatory effects of acute and repeated morphine treatments, *Pharmacol. Biochem. Behav.*, 60 (1998) 293–303.
- [14] Thomas, A.W., Kavaliers, M., Prato, F.S. and Ossenkopp, K.P., Pulsed magnetic field induced ‘analgesia’ in the land snail, *Cepaea nemoralis*, and the effects of mu, delta, and kappa opioid receptor agonists/antagonists, *Peptides*, 18 (1997) 703–709.
- [15] Yajeya, J., de la Fuente Juan, A., Merchan, M.A., Riobos, A.S., Heredia, M. and Criado, J.M., Cholinergic responses of morphologically and electrophysiologically characterized neurons of the basolateral complex in rat amygdala slices, *Neuroscience*, 78 (1997) 731–743.